

Recognition of pathogenically relevant house dust mite hypersensitivity in adults with atopic dermatitis: A new approach?

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Background: The pathogenic importance of the ubiquitous house dust mite, *Dermatophagoides pteronyssinus* (Dp), in atopic dermatitis is unclear.

Objective: We aimed to explore the relevance of Dp hypersensitivity in adult patients with atopic dermatitis by using an in vivo topical challenge method and in vitro assays for T-cell reactivity.

Methods: Dp and control skin prick test solutions were applied to the cubital fossae of 20 patients twice daily for 4 days; the severity of dermatitis and pruritus in the challenge sites were determined before and after testing. The same solutions were used in PBMC proliferation assays that included 10% fresh, autologous serum, the latter aimed at maximizing IgE-facilitated allergen presentation.

Results: Although most patients had markedly elevated Dp-specific serum IgE levels, only 6 of 20 patients developed increases in cubital fossa dermatitis severity and pruritus scores that were greater at sites of application of Dp solution than at control sites. In addition, PBMC proliferation in response to Dp solution in the presence of autologous serum was significantly greater in the in vivo challenge-positive patients than in those who did not respond to challenge. A subgroup of patients (7/20) also developed transient but pronounced contact urticaria at sites of Dp application.

Conclusion: These findings suggest that hypersensitivity to Dp might be clinically relevant in approximately one third of the adult atopic dermatitis population studied. They also point to methods of identifying patients who might respond to house dust avoidance measures. (*J Allergy Clin Immunol* 2002;109:1012-8.)

Key words: Atopic dermatitis, IgE-facilitated allergen presentation, T lymphocytes, *Dermatophagoides*, hypersensitivity, clinical relevance

The role of environmental allergens in the pathogenesis of atopic dermatitis (AD) remains a controversial subject, opposing views sometimes being expressed by allergists and dermatologists. For example, a definition published recently in a major textbook of dermatology does not include the word *allergy*, and it is stated that the IgE anti-

Abbreviations used

AD: Atopic dermatitis

Dp: *Dermatophagoides pteronyssinus*

FAP: Facilitated allergen presentation

SASSAD: Six area, six sign atopic dermatitis

bodies present in the blood often appear incidental to the disease.¹ Indeed, in many dermatology departments allergen avoidance plays little part in the management of AD. In contrast, the presence of allergen-specific IgE is sometimes taken by other researchers to indicate a specific role for the respective allergen in disease pathogenesis and to form the basis for management by specific allergen avoidance.² However, rigorous allergen avoidance can be difficult to achieve, especially with respect to the house dust mite, *Dermatophagoides pteronyssinus* (Dp), and when investigated in a controlled manner in patients with AD, avoidance has yielded unpredictable and/or disappointing results.²⁻⁵ In several other studies, the levels of allergen-specific serum IgE and responses to epicutaneous allergen patch testing have been determined in patients with AD in attempts to identify causal factors, but reliably predictive provocation assays for use in planning allergen avoidance have not yet been firmly established.⁶ Scepticism about the pathogenic importance of IgE-mediated events in AD also arises from the evidence that the immediate, transient, IgE-dependent weals commonly induced by allergen skin prick testing bear little resemblance to the typically chronic, T cell-rich skin lesions.¹ However, a pathogenic role for allergen-specific IgE in chronic atopic disease has been suggested by the evidence for IgE-facilitated allergen presentation (FAP), in which IgE bound to high- or low-affinity Fcε receptors on antigen-presenting cells acts as an allergen-focusing structure, facilitating allergen presentation and resultant T-lymphocyte activation by up to 1000-fold.⁷⁻¹⁰ This process was shown under certain in vitro experimental conditions to be mediated by low-affinity IgE receptors (CD23) on B lymphocytes^{7,10} and by high-affinity IgE receptors (FcεRI) on monocytes⁸ and blood dendritic cells.⁹

We have therefore used a modification of an in vivo unoccluded topical allergen provocation method,¹¹ in combination with in vitro PBMC proliferation assays in which the potential for IgE-FAP is maximized, in attempts to identify a subgroup of adult patients with AD who have clinically relevant hypersensitivity to Dp.

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Funded in part by the National Eczema Society.

Received for publication October 17, 2001; revised February 26, 2002; accepted for publication March 6, 2002.

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0091-6749/2002 \$35.00 + 0 1/85/124654

doi:10.1067/mai.2002.124654

TABLE I. Patient details and results of IgE assays

Patient no.	Age (y)	Sex	Duration (y)	History*	SASSAD score	Dp prick test† (mm)	Dp IgE‡ (kU/L)	Total IgE§ (kU/L)
1	20	F	20	A	35	10	>100	>1000
2	20	F	20	A	14	12	>100	>1000
3	29	M	29	R	13	10	>100	>1000
4	35	M	20	R	4	0	0.9	>1000
5	30	F	28	A	16	5	>100	782
6	22	F	21	A/R	19	15	>100	>1000
7	62	F	62	A/R	6	0	16.3	>1000
8	21	M	21	A	17	10	>100	>1000
9	32	M	32	A/R	11	0	14.3	>1000
10	32	M	30	A/R	12	5	69.7	517
11	46	M	45	A/R	5	8	>100	914
12	25	M	25	A/R	32	13	>100	>1000
13	19	F	19	A/R	5	15	>100	>1000
14	17	M	17	A	23	8	>100	>1000
15	37	M	37	A/R	12	20	>100	>1000
16	51	M	34	A	29	15	>100	>1000
17	21	M	20	A/R	12	15	60.3	>1000
18	50	M	50	A	23	15	>100	>1000
19	25	M	25	A/R	23	4	>100	>1000
20	27	M	27	A/R	35	0	2	>1000

SASSAD, Six area, six sign atopic dermatitis; Dp, *Dermatophagoides pteronyssinus*; A, asthma; R, allergic rhinitis.

*Personal history of asthma or allergic rhinitis.

†Largest weal diameter. Control skin prick test results were negative in each case except that of patient 13, in whom the control weal measured 5 mm.

‡Levels of 0.9 and 2 are classified as weakly positive, those of 14.3 and 16.3 as positive, and those above 60 as strongly positive.

§Normal range is up to 122 kU/L.

METHODS

Patients

After local research ethics committee approval of the project, 20 informed, consenting adults, each aged at least 17 years, with AD meeting standard diagnostic criteria¹² were recruited from the dermatology clinic of the Leicester Royal Infirmary (Table I). The patients were receiving conventional treatment, including topical steroids of varying potency, and emollients. Those who had received systemic immunosuppressives (including oral corticosteroids, cyclosporine, or azathioprine) or UV phototherapy in the preceding 2 months were excluded, but patients were otherwise recruited randomly. Patients had active disease of varying severity (Table I), as quantified by the six area, six sign atopic dermatitis (SASSAD) scoring system,^{13,14} in which 6 different skin areas can each be assigned a maximal score of 3 per sign, giving a maximal score of 18 per area and 108 overall.

Skin challenge

Skin prick testing was carried out through use of standard methods with commercial Dp solution (10,000 allergy units per milliliter; HAL, Haarlem, The Netherlands). Each 1 mL of the Dp solution and each 1 mL of the control solution contained 5 mg phenol, 6.6 mg ϵ -aminocaproic acid, 4.6 mg disodium hydrogen phosphate, and 0.6 mg sodium dihydrogen phosphate dissolved in glycerol/water (1:1 by volume). The same skin prick testing solution and control (HAL) were used for topical challenge. One drop (approximately 50 μ L) of Dp or control solution was applied to the cubital fossae of each patient and spread over a 5 $\times$ 5-cm area with the rounded end of a cleaned polypropylene test tube dedicated for use with the correspondingly coded solution. The spread solutions were allowed to dry (for approximately 5 minutes), and the procedure was repeated twice daily for 4 days. The first applications were carried out by the investigator, and each patient was then observed

for at least 30 minutes. Subsequent applications were carried out by the patients, who returned after 4 days for assessment. Although the Dp solution was straw-colored and the control solution colorless, double-blinding was attempted through the use of opaque dropper bottles and a randomized coding system. The severity of dermatitis in the patients' cubital fossae was scored by the same investigator before and after the 4-day challenge on a scale of 0 to 3 for each of 6 features (erythema, excoriation, dryness, fissuring, exudation, and lichenification), as in the SASSAD system,^{13,14} giving a maximum of 18 for each cubital fossa score. The pruritus in each cubital fossa was also scored before and after 4-day challenge by means of 100-mm linear analog scales on which 0 indicated the absence of itching and 100 the presence of the worst itch imaginable. Patients avoided oral antihistamines and application of topical steroids to the cubital fossae for 5 days before and during challenge but were permitted use of bland emollients.

Preparation of serum and PBMC proliferation assays

Immediately before skin prick testing or topical challenge, venous blood was taken for preparation of PBMCs and serum. Aliquots of serum were frozen, stored at -80°C , and subsequently used for measurement of total IgE and Dp-specific IgE (Millenia Microplate Kinetic ELISAs, EuroDPC, Glyn Rhonwy, United Kingdom). Fresh serum was also used immediately for supplementation of autologous PBMC cultures. PBMCs were prepared by density gradient centrifugation on Lymphoprep (Nycomed, Oslo, Norway). Washed interface cells (6×10^5 per milliliter) were cultured for 5 days in triplicate in RPMI-1640 medium containing either 10% (v/v) fresh, unheated autologous serum or 10% heat-treated (56°C for 60 minutes) allogeneic human AB serum (Sheffield Blood Transfusion Service, Sheffield, United Kingdom). A wide range of dilutions of unmodified Dp or control skin prick test solutions (HAL), equivalent



FIG 1. Effects of controlled topical application of Dp allergen solution. **A**, Increased dermatitis after 4-day application of Dp solution to the left cubital fossa of patient 19. **B**, No immediate response after the first application of control solution to the right cubital fossa of patient 17. **C**, Immediate contact urticaria after the first application of Dp allergen solution to the left cubital fossa of patient 17.

to at least 0.04 to 40 $\mu\text{L/mL}$, were added for the full duration of each culture. [Methyl- ^3H]-thymidine (1 μCi per well; Amersham Pharmacia, Amersham, United Kingdom) was added for the last 6 hours of the assay, cells were harvested, and cellular radioactivity was determined by liquid scintillation counting. Cell proliferation is expressed in counts per minute or as stimulation indices (cpm in the presence of Dp divided by cpm from cultures containing the same proportion of control solution).

Preparation and culture of CD4⁺ cells

In experiments to determine the main subset of cells among PBMCs that proliferated in response to Dp solution, CD4⁺ T cells were purified from PBMCs by positive selection with Dynabeads (Dyna, Oslo, Norway) to yield a population of cells that were >98% pure, as previously described.¹⁵ The purified CD4⁺ cells (2×10^5 per milliliter) were incubated for 5 days with autologous irradiated (20 Gy) PBMCs (5×10^5 per milliliter) in medium containing 10% autologous serum and a range of concentrations of Dp solution. After positive selection of CD4⁺ cells from PBMCs with Dynabeads, the CD4⁺ population (6×10^5 per milliliter) was also incubated for 5 days under otherwise identical conditions. Thymidine incorporation was determined as described above. The cells used in these experiments were from a patient with AD whose PBMCs had previously been shown to respond to Dp.

RESULTS

Skin challenge

For each patient, age, sex, duration of AD, personal history of other atopic disorders, and SASSAD score for general severity, as well as results of skin prick testing and Dp-specific and total serum IgE measurements, are shown in Table I. All 20 patients had markedly elevated total serum IgE levels. Sixteen of the 20 patients also had strongly positive Dp-specific IgE levels as measured by ELISA, whereas 2 patients' levels were classified as positive and 2 others' as weakly positive. Dp skin prick test results were positive in all patients except the 4 with lower Dp-specific IgE levels.

Six of the 20 patients showed increases in cubital fossa dermatitis severity (Fig 1) and pruritus scores that were greater at sites of Dp application than at control sites

after 4-day challenge (Table II). Seven patients developed immediate contact urticaria within a few minutes after the first application of Dp, associated with severe itching (Table II). No such reactions were seen at sites of control application. Reactions lasted 30 to 60 minutes (Fig 1). In all cases the urticarial reactions became less pronounced with repeated Dp challenge, and they could no longer be elicited by day 4. Four of the patients who developed immediate, transient urticaria also demonstrated increased dermatitis and pruritus scores in response to Dp, measured after 4 days (Table II).

PBMC and CD4⁺ T-cell responses

Clear, dilution-related PBMC proliferation in response to Dp was seen with 12 of the 20 PBMC samples cultured in the presence of fresh, autologous serum, whereas 8 samples were essentially unresponsive under these conditions. When present, proliferation was, with a single exception (patient 13), clearly greater in the presence of 10% unheated autologous (IgE-containing) serum than with 10% heat-inactivated, allogeneic AB serum (Fig 2 and Table III). Furthermore, when PBMC proliferation in the presence of 10% fresh, autologous serum was expressed as maximal stimulation indices, responses were shown to be significantly higher in the topical challenge-positive group than in the challenge-negative group ($P = .004$, Mann-Whitney test). Maximal stimulation indices were >25 in 5 of 6 samples from topical challenge-positive patients and <25 in 13 of 14 samples from patients who were challenge-negative (Table III).

Finally, experiments with CD4⁺ and CD4⁻ cell populations purified from Dp-responsive PBMCs through use of Dynabeads showed that the Dp-induced response was mainly due to proliferation of CD4⁺ cells (Fig 3).

DISCUSSION

In these studies, a commercial Dp solution was used for skin prick testing and topical challenge and, without modification, for in vitro PBMC proliferation assays,

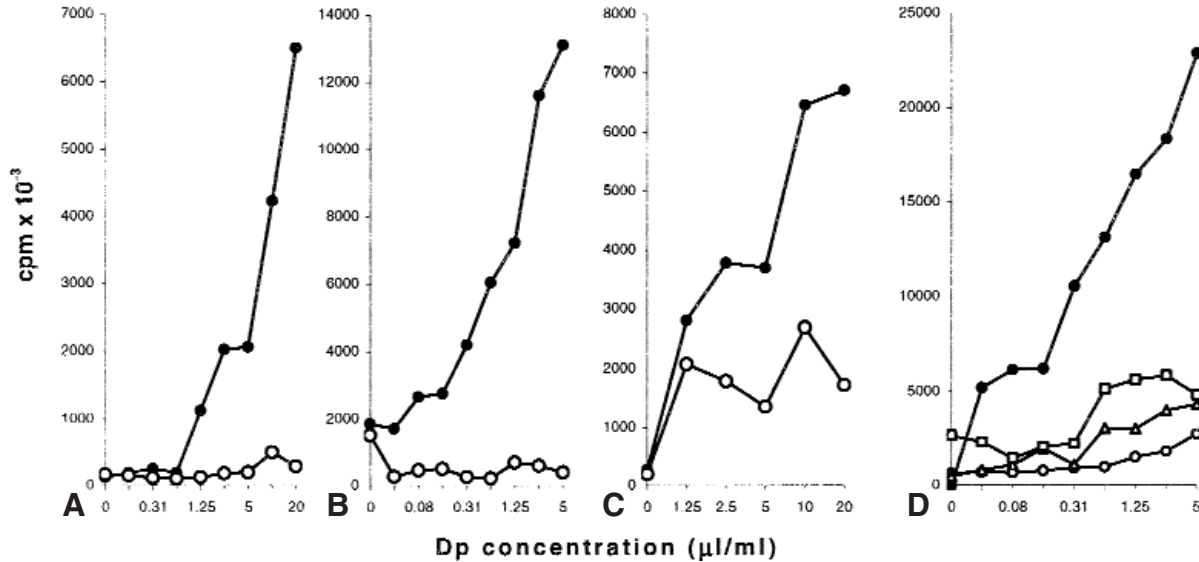


FIG 2. Representative PBMC proliferative responses to Dp in the presence of fresh, autologous serum (closed circles) and heat-treated allogeneic AB serum (open circles). Results with PBMCs from patients 5 (A), 14 (B), 19 (C), and 17 (D) are shown as examples. In D, 2 additional different batches of allogeneic AB serum (open triangles and open squares) were used for further comparison. The mean results of triplicate assays are shown. SEs were less than 30% of the means.

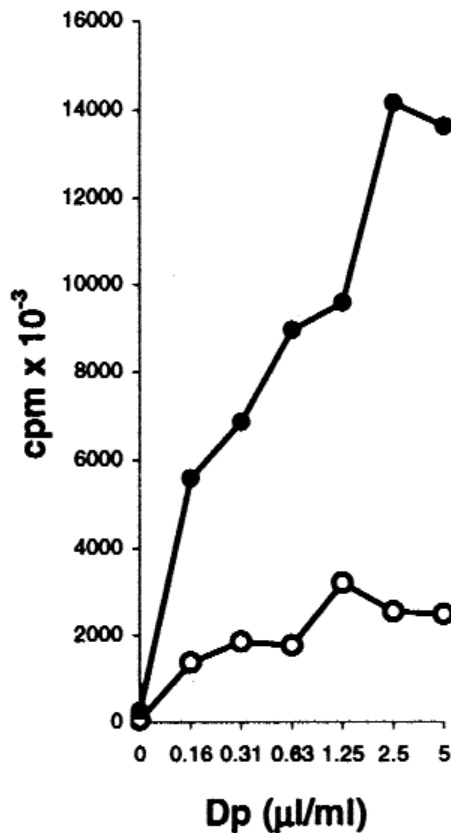


FIG 3. Responses to Dp solution of purified CD4⁺ cells in the presence of autologous, irradiated PBMCs as antigen-presenting cells (closed circles) compared with responses of CD4⁻ cells purified from the same PBMC population (open circles). The mean results of triplicate assays are shown. SEs were less than 30% of the means.

because direct comparisons of the effects of the same preparation were intended. The results obtained suggest that adult patients with AD who are likely to develop delayed, dermatitic responses to contact with Dp allergens might be identified by unoccluded topical challenge of the cubital fossa, a classic site of involvement in AD. However, the site-specificity of the responses to Dp application and the question of whether similar responses are seen in nonflexural areas have not been explored in the present work. Positive responses might be recorded by scoring the severity of dermatitis and quantifying local pruritus, both before and after 4 days of twice-daily challenge with a commercial Dp solution. Our findings suggest that the identification of these patients might be enhanced by the additional finding of pronounced PBMC responses to Dp (maximal stimulation indices, >25) when the 5-day proliferation assays are done in the presence of 10% unheated, fresh, autologous serum. The latter supplement might maximize IgE-FAP,⁷⁻¹⁰ a possibility supported by our ongoing work¹⁶ showing that (1) heating of autologous serum at 56°C for 60 minutes under conditions that irreversibly denature IgE^{17,18} or (2) the addition of FcεRI and CD23 blocking antibodies reduces atopic PBMC responses to Dp. That the described assay conditions are adequate to maximize IgE-FAP is also supported by experiments showing that preincubation of atopic PBMCs with undiluted, fresh, autologous serum did not enhance PBMC proliferation in response to Dp above that seen in the assays described in the current work (data not shown).

Our findings indicate that clinically detectable contact reactivity and concomitant strong *in vitro* PBMC proliferative responses to Dp allergens occur in only a minority (6/20) of adult patients with AD in the British East

TABLE II. Cubital fossa dermatitis and pruritus scores before and after 4-day topical challenge with Dp and control solutions and occurrence of immediate contact urticaria

Patient no.	Dp solution			Control solution			Contact urticaria
	Before	After	Δ	Before	After	Δ	
1	3/8	3/8	0/0	3/8	3/8	0/0	0
2	1/5	1/10	0/+5	2/5	1/0	-1/-5	0
3	0/1	0/1	0/0	0/1	0/1	0/0	+
4	0/1	0/1	0/0	0/1	0/1	0/0	0
5	<u>3/5</u>	<u>7/33</u>	<u>+4/+28</u>	<u>3/5</u>	<u>4/14</u>	<u>+1/+9</u>	0
6	5/4	5/12	0/+8	5/4	5/5	0/+1	0
7	0/0	0/0	0/0	0/0	0/0	0/0	0
8	2/2	2/2	0/0	2/2	2/2	0/0	0
9	0/2	0/2	0/0	0/2	0/2	0/0	0
10	0/3	0/3	0/0	0/7	0/5	0/-2	+
11	1/3	1/7	0/+4	1/16	1/1	0/-15	0
12	2/5	<u>6/67</u>	<u>+4/+72</u>	2/5	1/5	-1/0	0
13	0/4	0/9	0/+5	0/4	0/9	0/+5	+
14	<u>4/9</u>	<u>6/62</u>	<u>+2/+53</u>	<u>4/4</u>	<u>2/20</u>	<u>-2/+16</u>	+
15	<u>0/1</u>	<u>3/99</u>	<u>+3/+98</u>	<u>0/33</u>	<u>0/23</u>	<u>0/-10</u>	+
16	1/5	1/5	0/0	1/5	1/5	0/0	0
17	<u>1/1</u>	<u>2/15</u>	<u>+1/+14</u>	<u>1/1</u>	<u>1/5</u>	<u>0/+4</u>	+
18	2/1	2/1	0/0	1/1	1/1	0/0	0
19	<u>4/30</u>	<u>6/60</u>	<u>+2/+30</u>	<u>4/30</u>	<u>4/40</u>	<u>0/+10</u>	+
20	3/20	1/1	-2/-19	3/20	1/1	-2/-19	0

The cubital fossa dermatitis severity scores, as described in the Methods section, are shown in bold print on the left in each column, and the pruritus linear analog scores (in millimeters) are shown on the right in each column; shown are values before and after 4-day challenge with Dp or control solutions. The differences between respective scores before and after challenge are shown in the columns headed Δ. The underlined values are from the 6 patients (5, 12, 14, 15, 17, and 19) whose dermatitis severity and pruritus scores both increased in the cubital fossa to which Dp solution had been applied. Such increases either were not seen or were much less prominent in the opposite cubital fossae, to which control solution had been applied.

TABLE III. PBMC proliferation in response to Dp extract in the presence of fresh, autologous serum or heat-treated allogeneic AB serum

Patient no.	Fresh, autologous serum*	Heated allogeneic AB serum*
Topical challenge-positive patients		
5	69.9	4.1
12	26.4	3.2
14	47.4	1.2
15	12.9	4.2
17	100.3	22.1
19	36.8	9.7
Mean ± SD	49.0 ± 31.7†	7.4 ± 7.7‡
Topical challenge-negative patients		
1	16.9	9
2	1.4	1.3
3	2	2.5
4	2.8	2.4
6	16.7	2.8
7	6	2.8
8	9.2	3.4
9	23.2	2.1
10	7.8	4.9
11	4	3.1
13	56.5	54
16	3.5	2.5
18	4.1	2.4
20	1.4	1.4
Mean ± SD	11.1 ± 14.7	—

*Results are expressed as maximal stimulation indices and are the means of triplicate assays. PBMC cultures in which clear, dilution-related responses to Dp extract were seen are indicated in bold type.

†P = .004 in comparison with results of corresponding cultures from challenge-negative patients (Mann-Whitney test).

‡P = .006 in comparison with results from the same patients' cells cultured with fresh, autologous serum (paired t test).

Midlands region—this despite the fact that the large majority of these patients have strongly positive serum test results for Dp-specific IgE. The reasons for the discrepancy between the synthesis of allergen-specific IgE and PBMC responses to allergen in certain patients have not been elucidated in the present work and are the subject of further research. It can be speculated that the Dp-reactive, T_H2 -like cells that provide B-cell help in allergen-specific IgE synthesis¹⁹ might play a major role in this respect in the lymph node environment but that they might not be effective in directly provoking persistent Dp-mediated inflammatory reactions in peripheral tissues such as the skin and might not participate in Dp-mediated PBMC proliferation. This possibility is supported in part by the report that there was correlation neither between the levels of total serum IgE and the cytokine profiles of T cells infiltrating skin lesions in patients with AD nor between Dp-specific serum IgE levels and numbers of Dp-specific T-cell clones in lesions.²⁰ The proinflammatory role of T_H2 -like cells in the chronic skin lesions of AD has been a controversial subject, some authors previously stressing the relative importance of IL-4- and IL-5-producing T_H2 -like cells^{21,22} and others demonstrating a role for IFN- γ -producing non- T_H2 cells, especially in persistent AD lesions.²³⁻²⁵ We hypothesize that an appropriate repertoire of Dp-reactive, skin-seeking peripheral blood effector T cells, together with high levels of allergen-specific IgE that support FAP, is essential for the occurrence of Dp-induced delayed skin reactions in patients with AD. Our observations suggest that measurement of Dp-specific serum IgE alone is not a reliable indicator of dermatologically relevant Dp hypersensitivity, and this might explain the disappointing results of house dust avoidance in patients with AD who are recruited on the basis of IgE levels alone.² The findings also suggest a new approach to the identification of patients with AD who will respond to rigorous house dust avoidance; however, this possibility remains to be tested in clinical trials.

It is noteworthy that we were unable to induce dilution-related PBMC proliferation in response to Dp in 8 of 20 patients, even in the presence of autologous serum, and in half of those 8 patients maximal stimulation indices were less than 3 (Table III). Furthermore, we have been unable to induce significant PBMC proliferation with a wide range of concentrations of the Dp preparation in any of 7 normal adult volunteers aged 18 to 55 years (with negative personal and family histories of atopic disorders, normal/negative total and Dp-specific serum IgE levels, and negative Dp skin prick test results) in 5-day assays with 10% autologous serum (data not shown). These findings are in agreement with previous work reporting that approximately one third of Dp skin prick test result–positive atopic subjects and more than 90% of nonatopic controls did not demonstrate PBMC responses to Dp allergen,²⁶ but they do not support reports that the PBMCs of nonatopic individuals react strongly to Dp extracts.^{27,28} Further work is required to explain these critical differences.

We also show for the first time that Dp allergens cause pronounced contact urticaria in some patients with AD, and we suggest that this response might be relevant to the spectrum of symptoms and signs of AD. The diminished urticarial responses following repeated application of Dp to the same, initially reactive site are not unexpected, given that tachyphylaxis is likely to occur as a result of depletion of local mast cell histamine. The reason for pronounced contact urticaria in some patients but not in others is currently unexplained. Responses occurred in cubital fossae that were either involved with active AD or clinically normal (Table II). The ability of Dp solution to produce pronounced urticaria after application to clinically uninvolved atopic skin (Fig 1) indicates a surprising capacity of the allergens to be absorbed percutaneously in biologically relevant amounts. It is conceivable that the inherent proteolytic activity of Dp allergens²⁹ enhances their percutaneous absorption, though the rapidity of onset of the urticarial reactions following Dp application suggests that this mechanism might not function significantly in this setting.

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